Ionization Techniques of Volatile Compounds CI vs. EI

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Abstract

Different ionization modes were used in this study to identify unknown compounds. EI (electron impact) ionization was used at 70 eV energy level to create comparable mass spectra, therefore compound identification was done using NIST mass library. CI (chemical ionization), as a softer ionization, was used to avoid the hard fragmentation of molecules. Using CI, molecular ion can be found on the mass spectra, which molecular ion gives more information about the component. In our study lower EI energy level was used to create similar mass spectra as CI, so molecular ions were also preparated. The applied lower EI energy level warrants the undamaged molecule and can give more information about the analyzed molecule as well as CI. In untargeted analysis, it is very important to extract as much information about the component as possible to determine the molecule structure and identify it.

Introduction

Determination of volatile fungal metabolites usually is done using gas chromatography (GC) coupled with mass spectrometry (MS) methods. Solid-phase micro extraction (SPME) fibers are used worldwide to collect the volatile compounds from the headspace (HS) (1, 2, 3). It seems that the above mentioned coupled technique has enough high sensitive detection capabilities, however the identification often cannot be really completed, we can still find only chemical classes (for example terpenoids, sesquiterpenes, unidentified diterpenes…) (2). According to a usual workflow the fungal VOCs are detected and identified using single quadropole MS data in electron ionisation (EI) mode (3) and applying a similar evaluation process to identify EI spectra. In EI mode, ions are formed when a 70 eV beam of electrons hits the sample molecules in the gas phase so many fragment ions create. These ions can be used for determining the structure of the molecules; therefore we can use the generated spectrum to compare library spectra and identify the compounds. Unfortunately, some compounds will completely fragment and not give molecular ions. In these cases there is an alternate method, which gives a softer ionozation process. This method is the chemical ionozation (CI) mode.Untargeted analysis requires as much information about the molecules as possible to get better opportunity to identify the found component.

Experimental

Samples

Different mould samples were inoculated on PDA substrata in 20 mL HS-vials. These microorganisms were measured using HS-SPME-GC EI/CI qTOF MS in EI and CI mode.

Different tomato volatiles were sampled from 20 mL HS-vials and analyzed by HS-

SPME-GC-MS. Only EI mode was used but EI energy level was modified.

Equipment

EI measures: An Agilent 6890 Gas Chromatograph coupled with a 5975 C MSDMass Spectrometer was used with a non-polar HP-5MS ((5 %-phenyl)-methylpolysiloxane; 30 m, 0.25 mm i.d., 0.25 μm film, Agilent Technologies) column. Optimised oven temperature program was used [4]. During the procedures, inlet temperature was held constant at 250 °C. Hydrogen was used as carrier gas with a constant 1.2 mL/min flow to accelerate the separation on the column. The MS source temperature was set to 230 °C and the quadrupole temperature was held at 150 °C. Positive electron ionisation (EI+) was used, with an electron energy level of 70 eV. After the energy level was decreased until it reached 5 eV (the minimum E level of mass spectrometer). The MS was tuned using perfluorotributylamine (PFTB) every day before the measurements. Agilent Enhanced MSD ChemStation software handled the GC and MS parameters.

EI and CI measures: Agilent 7890B GC and Agilent 7200 EI/CI qTOF MS coupled analytical system were used to analyse the emitted compounds. HP-5MS capillary column (the same as in EI measures) and 5 m long pre-column was equipped without stationary phase. Helium was used as a carrier gas with 1.3 ml/min (pre-column 1.2 ml/min) gas flow. Oven temperature program was set according to Stoppacher and his co-worker (Soppacher et al., 2010), but we supplemented the program after the last step, thus the following oven temperature program was achieved: 40 °C (hold 2 min), 10 °C/min to 200 °C, 25 °C/min to 260 °C (hold 5 min), 25 °C/min to 300 °C (hold 5 min).

EI TOF MS parameters: electron impact ionization (EI) at 70 eV, ion source 230 °C, quadrupole 150 °C, acquisition parameters: m/z range: 40-700, rate: 3.33 spectra/sec, transfer line to MS 280 °C.

CI TOF MS parameters: chemical ionization (CI) with 20% methane (gas flow 5 ml/min) at 160 eV, emission voltage: 10 μA, ion source 150 °C, quadrupole 150 °C, acquisition parameters: m/z range: 50-700, rate: 3.33 spectra/sec, transfer line to MS 280 °C.

Agilent Enhanced Data Analysis, Agilent MassHunter Qualitative Analysis B.07.00 and Agilent MassHunter Quantitative Analysis B.06.00 software were used for evaluation and comparison of the chromatograms. Mass Frontier software was used to fragment the compounds based on their molecule structure. The collected compounds were identified using NIST Mass Spectral Search Program (NIST 2011, Wiley 10th edition) and the calculated retention index was compared with literature values (NIST Chemistry WebBook).

Results and discussion

As a first step, samples were measured in EI mode using 70 eV energy level. The obtained compound's mass spectra were compared with reference mass spectra (NIST library); hence the identification step was done. To ensure the reliability of the result, different ionization modes were used. In the case of mould samples, beyerene compound was found at 19.44 min. Fig. 1. shows the results of EI and CI ionization. Using CI, molecular ion ($[M+H]^+$) was clearly found at 273.2578 m/z value and the mass accuracy was less than 0.5 ppm (TOF MS has higher resolution so mass accuracy is appropriate). Quasi molecular ion group ($[M-H]^+$, $[M]^+$, $[M+H]^+$) can also

found on the mass spectra which gives information about the molecule mass, in this case: 273.2578 m/z.

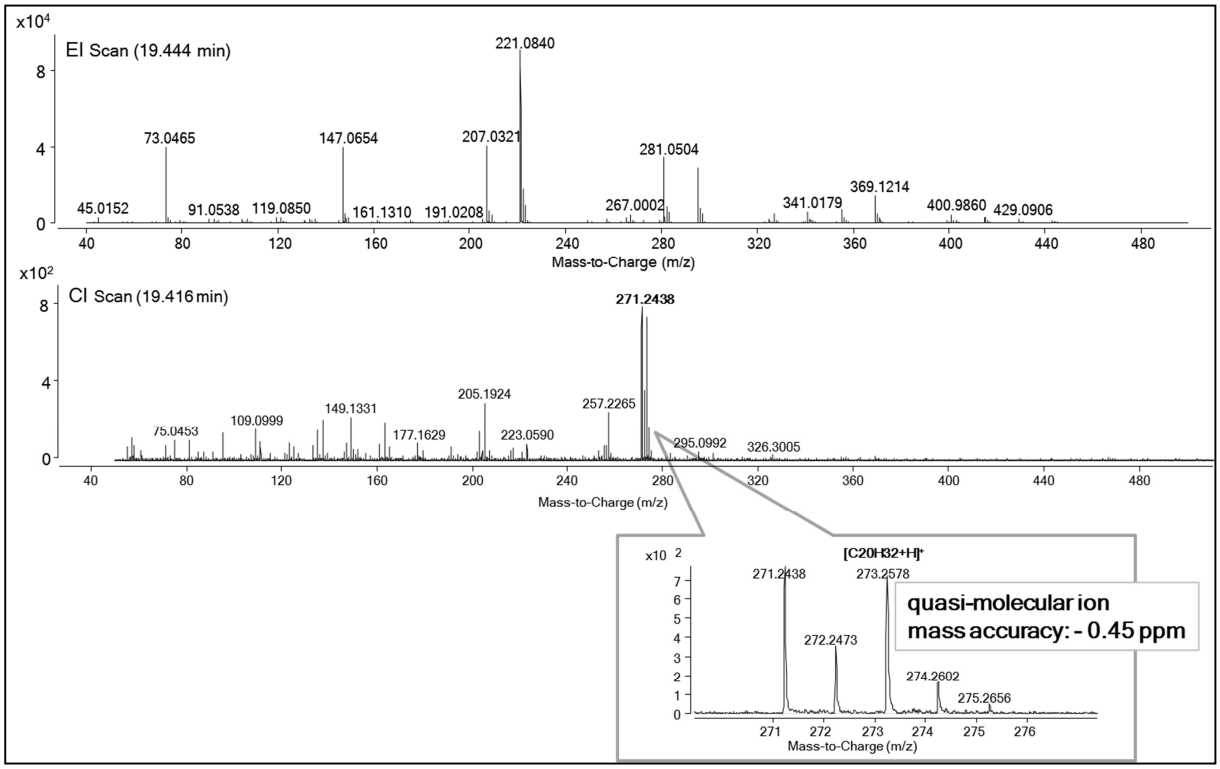

Figure 1. Example of EI vs CI mass spectra using TOF MS (mould sample). Mass spectra of beyerene: molecular weight: 272.247, molecular formula: C20H32, RI: 1898.4, ID: 85,2%

Using CI ionization, molecule identification could be more accurate because ionization is softer than in the case of EI; the molecule ion did not break into fragments. We can also avoid the fragmentation of molecular ion in EI mode due to decreasing EI energy level. Seven ionization energy levels were used in this experiment (70 eV, 60 eV, 50 eV, 40 eV, 20 eV, 10 eV, 5 eV). According to the results, the highest compound intensity was found at 60 eV energy level. Until 10 eV energy level, the fragmentation is very similar as it was at 70 eV. Using 5 eV ionization energy level, the molecular ions (M^+) appeared in the case of most compounds. After that we were able to compare the identified compound mass (identification step was done by NIST library using 70 eV) with the molecular ion mass which was created at 5 eV energy level. Fig 2. shows the experiment results and the clearly identified molecular ion.

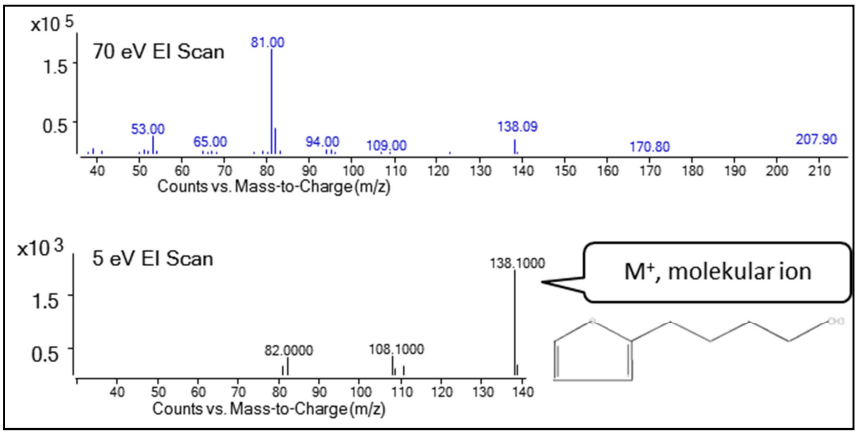

Figure 2. Example of decreased energy level of EI ionization using quadrupole MS (tomato sample). Mass spectra of furan, 2-pentyl-: molecular weight: 138.104, molecular formula: C9H14O, RI:1040, ID: 93.9%

Using softer EI ionization similar results were obtained as in the case of CI. Our EI method is also included the advance of CI mode, keeping the advances of EI, for example it is more widespread, easier to use and more understable than the CI.

Conclusion

In the case of decreased energy level of electron ionization, similar clear mass spectra can be reached as in the case of CI. Furthermore, molecular ion can be also found in 5eV energy level EI mass spectra. Using this ionization technique, more information can be evaluated from the analyzed molecule, which is beneficial, because untargeted analysis requires as much information about the molecules as possible to get better opportunity to identify the found, unknown component.

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